Mucin-like Antigens in a Human Pancreatic Cancer Cell Line Identified by Murine Monoclonal Antibodies SPan-1 and YPan-1

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ABSTRACT

The antigenic determinant recognized by monoclonal antibody SPan-1 is greatly elevated in sera of patients with pancreatic cancer but not in sera of normal individuals. Here we describe the mucin-like characteristics of the SPan-1 antigen isolated from culture medium and xenografts of the human pancreatic cancer cell line SW-1990. YPan-1, another pancreatic cancer associated monoclonal antibody, also reacts with the SPan-1 antigen. The SPan-1/YPan-1 antigens have densities of 1.4-1.5 g/ml and elute in the void volume of Sepharose CL-2B columns. They are resistant to degradation by chondroitinase ABC, nitrous acid, and hyaluronidase but susceptible to protease digestion and reductive \( \beta \)-elimination. All these characteristics suggest that the SPan-1 and YPan-1 determinants are carried on mucinous antigens. Both SPan-1 and YPan-1 immunoreactivities are unaffected by boiling or by alkaline and reduction of the mucins while they are abolished by mild periodate oxidation or neuraminidase and are markedly decreased by wheat germ agglutinin. Thus, their antigenic determinants are composed principally of carbohydrates with sialic acid, an absolute requirement for reactivity. However, the epitope specificities of SPan-1 and YPan-1 are different since YPan-1 does not compete with SPan-1 for binding to antigen. Moreover, YPan-1 and SPan-1 can be distinguished from several other sialic acid requiring, cancer associated antibodies such as B72.3, CSELEX-1, DU-PAN-2, OC-125, and 19-9 by their epitope characteristics or their tissue reactivity patterns.

INTRODUCTION

Immunological detection of mucin-like high molecular weight glycoproteins has become increasingly important in the diagnosis of various cancers, including cancer of the pancreas (1-5). We have previously reported (6) the production of a monoclonal antibody, YPan-1, against the human pancreatic cancer cell line Capan-2, which reacts with glycoproteins released by these cells into their culture medium (7). YPan-1 had a 99%, 90%, and 46% positive immunoreactivity with formalin fixed cancerous pancreas, stomach, and colon, respectively (6). Although YPan-1 reacts with normal pancreas it has little or no reaction with normal colon or stomach. In well differentiated pancreatic carcinoma, YPan-1 reacted with both the luminal border of cells and luminal contents. Another monoclonal antibody, SPan-1 (8), was recently produced in our laboratory using a high mucin containing pancreatic cancer cell line, SW-1990 (9), as the immunogen. Immunohistochemical studies using SPan-1 showed 89, 67, and 62% positive reaction with cancers of the pancreas, stomach, and colon, respectively. SPan-1 also reacts with normal pancreas but minimally with normal colon or stomach. Furthermore, 93% of sera from pancreatic cancer patients had elevated levels of the SPan-1 deter-

3 The abbreviations used are: PBS, phosphate buffered saline; BSA, bovine serum albumin; ELISA, enzyme linked immunosorbent assay; HPLC, high performance liquid chromatography; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; WGA, wheat germ agglutinin.
ograft mucins was used was 2 μg hexose/ml unless otherwise specified. Lectins were preincubated with antigen for 2 h at room temperature. WGA and succinylated WGA (Triticum vulgaris) were obtained from Vector Laboratories, and fucose binding protein from Lotus tetragonolobus was purchased from ICN ImmunoBiosciences. All other lectins were obtained from Sigma. In the competition ELISA, antibody was preincubated with inhibitor overnight at 4°C. A range of antibody concentrations was tested to determine the appropriate subsaturating levels.

Double Determinant Radiolmmunoassay. The procedure was similar to that described elsewhere (8). In competition studies the inhibiting antibodies were incubated with antigen for 2 h at 37°C prior to addition of radiolabeled SPan-1 or YPan-1. Partially purified monoclonal antibody 19-9 (4.8 mg/ml) was a gift from Dr. V. Zurawski, Jr. (Centocor, Inc.). DU-PAN-2 ascitic fluid was a gift from Dr. Richard S. Metzgar, and CSLEX-1 ascitic fluid was a gift from Dr. Paul I. Terasaki. Purified myeloma IgM was a gift from CalTag Laboratories.

Chemical and Enzymatic Treatments. After purified SPan-1/YPan-1 antigens from culture media or xenograft were treated with 0.05 M NaOH and 1 M NaBH4 at 37°C for 16 h, they were neutralized and desalted, and the borate was removed according to the method of Edge et al. (12). Mucins were also reduced and alkylated (13) or treated with nitrous acid (14) or periodate (15). The following enzymatic conditions were used: (a) bovine testicular hyaluronidase (0.5 mg/ml in citrate buffer, pH 5.5); (b) Clostridium perfringens neuraminidase (type X, 0.25 unit/ml in PBS, 2 h, 37°C); (c) Proteus vulgaris chondroitinase ABC (0.5 unit/ml in 0.05 M Tris-acetate buffer, pH 7.3); and (d) Streptomyces griseus Pronase E (protease type XIV, in 0.10 M Tris-CI containing 0.05 mM CaCl2, pH 8.0) at a 1:100 protease:mucin protein ratio. All enzymes were obtained from Sigma. Enzyme digests (37°C overnight unless otherwise stated) were boiled for 10 min prior to testing with antibody. As positive controls hyaluronidase and chondroitinase ABC were shown to be active against their respective substrates under the conditions used.

Immunoprecipitation. Protein A-positive Staphylococcus aureus (Boehringer Mannheim Biochemicals) were prewashed three times with Buffer A (0.5 mM NaCl, 1 mM EDTA, 0.1 mM PMSF, 0.1% BSA-1% Nonidet P-40 in 10 mM Tris, pH 8.0 (16) and then resuspended in Buffer A (10% original dry w/v). Radiolabeled medium antigens prepared as described above in PBS containing 0.1 mM PMSF were diluted 1:1 with Buffer A to a final volume of 400 μl. After nonspecific precipitation (incubation with 100 μl of washed Protein A for 1 h on ice with occasional shaking followed by centrifugation for 2 min at 12,000 × g in a microcentrifuge) the supernatant fluid was incubated successively with: (a) YPan-1, SPan-1, or myeloma (P3-X63-Ag 8.653 plus IgM (Zymed Laboratories) diluted 1:500 in the BSA solution for 90 min; and (d) "I labeled Protein A (106 cpm/ml) in the BSA solution and CSLEX-1 ascitic fluid was a gift from Dr. Paul I. Terasaki. Purified myeloma IgM was a gift from CalTag Laboratories.

Chemical and Enzymatic Treatment of Medium Antigens. [3H] Glucosamine labeled material released by SW-1990 cells into culture medium eluted principally at the excluded volume of Sepharose CL-2B columns (Fig. 1). The void volume fraction intensifying screen [Cronex Lighting Plus, DuPont (20)], in the case of 125I, at -70°C. For fluorography dried gels were exposed to untreated film without a screen. Proteins and glycoproteins were visualized by staining the gels with 0.25% Coomassie Brilliant Blue R-250 or periodic acid-Schiff (21). The following proteins were used for molecular weight standards: myosin, β-galactosidase, BSA, and ovalbumin (Sigma).

Affinity Chromatography on WGA-Agarose. Purified xenograft mucin was applied to 1-ml columns of WGA-agarose (Sigma), equilibrated with PBS, and eluted with increasing concentrations of N-acetylglucosamine in PBS. One-ml fractions were collected.

Chemical Analyses. Protein concentration was determined by the method of Lowry et al. (22) using BSA as standard. Hexose concentration was determined by the phenolsulfuric method (23) with galactose as standard. O-Linked oligosaccharide was determined by the fluorescent method of Crowther and Wetmore (24), using a Perkin-Elmer LS-2 filter fluorimeter at an excitation wavelength of 340 nm and an emission wavelength of 383 nm.

RESULTS

Enzymatic and Chemical Treatment of Medium Antigens. [3H] Glucosamine labeled material released by SW-1990 cells into culture medium eluted principally at the excluded volume of Sepharose CL-2B columns (Fig. 1). The void volume fraction

![Fig. 1. Effect of chondroitinase ABC on radiolabeled medium antigen.](cancerres.aacrjournals.org)
also contained most of the SPan-1 and YPan-1 immunoreactivity as determined by ELISA. Low levels of radioactivity, YPan-1 antigen, and SPan-1 antigen were detected in fractions at or just before the included volume. Extracellular high molecular weight glycoconjugates could be either glycoproteins or proteoglycan. However, the elution profiles of \(^{3}H\)glucosamine labeled material, of SPan-1 antigen, and of YPan-1 antigen were unaffected by pretreatment of the medium with chondroitinase ABC (Fig. 1; YPan-1 data not shown), which hydrolyzes chondroitin, chondroitin sulfates, hyaluronic acids, and dermatan sulfate (25). Lack of digestion by bovine testicular hyaluronidase (Fig. 2) further substantiated the conclusion that hyaluronic acids were not responsible for the void volume radioactivity or immunoreactivity (YPan-1 not shown). Similar results were obtained when medium antigens were treated with nitrous acid, ruling out the possibility of heparan sulfate (14). Rechromatography of the void volume fractions on another Sepharose CL-2B column in the presence of 4 M guanidine-HCl, to disassociate any high molecular weight proteoglycan aggregates (26), did not alter the elution profile of labeled molecules or immunoreactivities. However, when medium was digested with neuraminidase (Fig. 2), much of the radioactivity in the void volume disappeared with the appearance of a low molecular weight radioactive peak. Immunoreactivity with SPan-1 and YPan-1 (YPan-1 not shown) was completely abolished by sialic acid removal, concomitant with marked reduction in radioactivity.

Density of Medium Antigens. When the void volume fractions from Sepharose CL-2B columns containing the most radioactivity and SPan-1/YPan-1 immunoreactivities were pooled and centrifuged in the presence of CsCl, the radioactivity was found predominantly at a density of 1.4–1.5 g/ml (Fig. 3). The same fractions also contain the main portion of both SPan-1 and YPan-1 immunoreactivities.

β-Elimination and Protease Digestion of SPan-1 and YPan-1 Medium Antigens. When medium antigens purified by gel filtration and density gradient centrifugation were subjected to alkaline borohydride treatment to release O-linked oligosaccharides by β-elimination, the radioactivity was completely displaced from the void volume of Sepharose CL-2B to the included volume (Fig. 4). Immunoreactivity with SPan-1 was not detectable by direct binding ELISA but was detected in a competition ELISA, indicating that released oligosaccharides did not bind to microtiter plates but were recognized by antibody. Those fractions containing the greatest amounts of radioactivity proved also to be the most inhibitory to SPan-1 (Fig. 4). Similarly, when purified SPan-1 antigens were digested with Pronase E, immunoreactivity was detected only with the competition ELISA (Fig. 4). After 24 h of enzymatic hydrolysis, most of the radioactivity and SPan-1 inhibitory activity were shifted toward the included volume. In contrast to the results of alkaline borohydride treatment, both \(^{3}H\) and SPan-1 inhibitory activity were broadly distributed between the void volume and the included volume, indicating the presence of immunoreactive glycopeptides of heterogeneous sizes. In the case of YPan-1, it was not possible to detect antigenic activity after either β-elimination or protease hydrolysis.

Isolation of SPan-1 and YPan-1 Antigens from SW-1990 Xenografts. We also carried out similar studies using SW-1990 xenografts grown in athymic nude mice. As shown in Table 1, both SPan-1 and YPan-1 antigens were present in CsCl fractions corresponding to densities of 1.4–1.5 g/ml, a result similar
Conventional column chromatography to isolate the high molecular weight antigens. As shown in Fig. 5, YPan-1 and SPan-1 immunoreactivities and O-linked oligosaccharides were found in the void volume region after treatment of xenograft antigen with alkaline periodate. Neuraminidase abolished reactivity of both antibodies. Neuraminidase pretreatment also destroyed all SPan-1 and YPan-1 reactivities. These results suggest that the carbohydrates of the xenograft mucin with 0.1 M periodate reduced YPan-1 and SPan-1 activities by 38% and 12%, respectively, while 1 mM periodate abolished reactivity of both antibodies. Neuraminidase pretreatment also destroyed all SPan-1 and YPan-1 reactivities. Although YPan-1 immunoreactivity was decreased by both treatments, a low level of YPan-1 inhibitory activity was detectable with the competition ELISA using a lower antibody concentration than in the control (Fig. 6).

Comparison of Immunoprecipitated Media and Purified Xenograft Antigens by SDS-PAGE. When the [3H]glucosamine labeled high molecular weight fraction was immunoprecipitated with SPan-1 or YPan-1, 76-86% of the total label was recovered in the pellets. Under the same conditions, the myeloma ascites proteins used as control precipitated only 2-4% of the total counts. This result indicates that the bulk of the [3H]glucosamine labeled high molecular weight glycoprotein is recognized by SPan-1 and YPan-1. In agreement with the results of column chromatography (Fig. 1), [3H]glucosamine labeled glycoproteins immunoprecipitated with YPan-1 or SPan-1 did not penetrate into a 7% resolving gel (Fig. 7). Thus even under denaturing conditions and in the presence of mercaptoethanol, these antigens have molecular sizes substantially greater than 200,000, the largest molecular weight standard used (myosin). Similarly, after immunoblotting of purified xenograft antigen a substantial portion of SPan-1 and YPan-1 immunoreactivity remained in the 4% gel or, in the case of SPan-1, at the top of the 7% gel. No immunostaining was observed when myeloma ascites fluids were used in place of the first antibody. Periodic acid-Schiff reactive material was also observed in the 4% gel and at the top of the 7% gel.

General Characteristics of SPan-1 and YPan-1 Epitopes. The above results demonstrate that the YPan-1 and SPan-1 epitopes are present on mucin-like molecules both in spent medium and in xenograft tumors. Neither boiling nor reduction and alkyla-
dation of xenograft mucins had any effect on the reactivity of either SPan-1 or YPan-1. In contrast, pretreatment of xenograft mucin with 0.1 mM periodate reduced YPan-1 and SPan-1 immunoreactivity by 38% and 12%, respectively, while 1 mM periodate abolished reactivity of both antibodies. Neuraminidase pretreatment also destroyed all SPan-1 and YPan-1 reactivities. These results suggest that the carbohydrates of the xenograft antigens may be due to nucleic acids since this fraction has a maximum absorbance at 260 nm as compared to 280 nm for Fraction 6. Fraction 6 also contained the highest protein concentration (Fraction 1). Hexose was present in two major peaks. The high phenolsulfuric positive reaction of Fraction 8 may be due to nucleic acids since this fraction has a maximum absorbance at 260 nm as compared to 280 nm for Fraction 6. The high phenolsulfuric positive reaction of Fraction 8 may be due to nucleic acids since this fraction has a maximum absorbance at 260 nm as compared to 280 nm for Fraction 6.

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**Table 1** Characterization of density gradient fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>Immunoreactivity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.120</td>
<td>0.145</td>
<td>0.187</td>
<td>0.190</td>
<td>0.290</td>
<td>0.495</td>
<td>0.295</td>
<td>0.063</td>
</tr>
<tr>
<td>YPan-1</td>
<td>0.105</td>
<td>0.120</td>
<td>0.098</td>
<td>0.110</td>
<td>0.090</td>
<td>0.230</td>
<td>0.180</td>
<td>0.070</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.100</td>
<td>0.075</td>
<td>0.057</td>
<td>0.062</td>
<td>0.056</td>
<td>0.162</td>
<td>0.093</td>
<td>0.450</td>
</tr>
<tr>
<td>Hexose&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3</td>
<td>10.9</td>
<td>12.4</td>
<td>14.7</td>
<td>21.7</td>
<td>61.6</td>
<td>32.2</td>
<td>9.1</td>
</tr>
<tr>
<td>O-linked oligosaccharides&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.15</td>
<td>6.25</td>
<td>4.10</td>
<td>2.20</td>
<td>1.10</td>
<td>0.75</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Protein&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.20</td>
<td>1.26</td>
<td>1.28</td>
<td>1.32</td>
<td>1.35</td>
<td>1.40</td>
<td>1.49</td>
<td>1.52</td>
</tr>
<tr>
<td>Density&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.25</td>
<td>0.36</td>
<td>0.45</td>
<td>0.50</td>
<td>0.55</td>
<td>0.65</td>
<td>0.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by ELISA (absorbance at 415 nm).
<sup>b</sup> Determined by phenolsulfuric assay (mg/ml).
<sup>c</sup> Determined by fluorescence assay in the presence of NaOH (relative fluorescence units).
<sup>d</sup> Determined by assay of Lowry et al. (mg/ml).
<sup>e</sup> Determined gravimetrically (g/ml).
MUCIN-LIKE ANTIGENS OF SPan-l AND YPan-1

U. M. vs

CONTROL

\[ 0.4 \quad D \] 0
\[ 0.3 \] 0
\[ 0.2 \] 0
\[ 0.1 \] 0

FRACTION NUMBER

Fig. 5. HPLC of partially purified xenograft mucins. CsCl fractions containing the maximum amount of SPan-1 and YPan-1 immunoreactivities (usually with densities of 1.4-1.5 g/ml) were pooled and passed through a TSK-G (4000) SWG column (30 x 2.15 cm). Three-mi fractions were collected. Vf end of the included volume. YPan-1 and SPan-1 immunoreactivities were determined by a direct binding ELISA, and hexose was determined by the phenolsulfuric method. Bar, fractions which were pooled and concentrated for SDS-PAGE (Fig. 7).

Mucins were also preincubated with several lectins such as WGA, succinylated WGA, fucose binding protein (L. tetragonolobus), Bandeiraea simplicifolia (BS-II; N-acetylglucosamine), Arachis hypogaea (galactose; N-acetylgalactosamine), Ulex europaeus (fucose), and Dolichos biflorus (N-acetylgalactosamine) at concentrations up to 1 mg/ml in an attempt to block binding of SPan-1 and YPan-1 to specific monosaccharides. No significant effect was observed except in the case of WGA (Table 2). WGA inhibited binding by both SPan-1 and YPan-1. Since WGA is reported to bind both sialic acids and N-acetylglucosamine we also tried the succinylated form of the lectin which binds only N-acetylglucosamine (27). Succinylated WGA had much less of an inhibitory effect on the binding of SPan-1 and YPan-1 to mucin, suggesting that WGA inhibits SPan-1 and YPan-1 through competition for sialic acid and not N-acetylglucosamine.

Mild alkali treatment of xenograft mucin in the presence of borohydride produced a fraction which, after desalting and removal of borate, could partially inhibit SPan-1 and YPan-1 (Fig. 8). The recovered oligosaccharide fraction had a markedly reduced ability to inhibit the antibodies (especially YPan-1) when compared to a comparable amount of starting material (based on hexose determination).

Relationship of the SPan-1 and YPan-1 Antigenic Determinants. Purified YPan-1 did not specifically inhibit SPan-1 binding to purified xenograft mucins in a double determinant radioimmunoassay developed with purified SPan-1 antibody (Table 3). This indicates that the epitopes for the two are distinct.

Fig. 6. Effect of alkaline borohydride and protease on purified xenograft mucins. Purified xenograft mucins were incubated with 0.05 M NaOH and 1 mM NaBH₄ for 16 h at 37°C or with Pronase E in 0.05 M Tris-Cl containing 0.05 mM CaCl₂, pH 8.0, for 16 h at 37°C. Chromatography conditions are the same as those described in legend to Fig. 2. YPan-1 and SPan-1 immunoreactivities were determined by competition ELISA.

Fig. 7. Comparison of immunoprecipitated radiolabeled media antigens with immunoblots of xenograft antigens. Radiolabeled medium immunoprecipitated as described in "Materials and Methods" and HPLC purified xenograft mucin (indicated by the bar in Fig. 5) were subjected to SDS-polyacrylamide electrophoresis using a discontinuous 4%/7% gel system. Xenograft antigens were then transferred electrophoretically to nitrocellulose paper for immunostaining with mouse monoclonal antibodies (see "Materials and Methods"). Periodic acid-Schiff staining was done directly on the gels. Medium antigen II) immunoprecipitated with SPan-1 (Lane 1: 21,200 cpm, developed 14 days) and YPan-1 (Lane 2; 16,820 cpm, developed 14 days). Xenograft antigen (A) immunoblotted with SPan-1 (Lane 3) and YPan-1 (Lane 4) or PAS stained (Lane 5). Molecular weight markers are ovalbumin (M, 45,000), BSA (M, 66,000), β-galactosidase (M, 116,000), and myosin (M, 205,000), K, thousands.

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significant amount of radiolabeled SPan-1 becomes bound if either purified YPan-1 or SPan-1. Different dilutions of purified xenograft mucin with unlabeled YPan-1 (heterologous) is used as the "catcher." Simultaneously, radiolabeled YPan-1 is also bound if unlabeled SPan-1 is used first as the catcher. The amount of radioactivity is a function of antigen level and requires at least 0.1 \( \mu \)g/well of either unlabeled antibody to be added as catcher. In an attempt to dissociate YPan-1 immunoreactivity from that of SPan-1, purified xenograft mucins were separated by affinity chromatography on WGA-lectin columns. No SPan-1 or YPan-1 antigen was eluted until 0.1 M N-acetylglucosamine was used. Significant amounts of both SPan-1 and YPan-1 immunoreactivities eluted from the column in parallel when a step gradient of N-acetylglucosamine (0.1, 0.2, 0.3, 0.4, and 0.5 M) was used. Thus, the two antigenic determinants were not dissociable by this method.

Comparison of SPan-1 and YPan-1 with CSLEX-1, DU-PAN-2, and 19-9. In addition to SPan-1 and YPan-1, SW-1990 xenograft mucin was recognized by three other sialic acid dependent monoclonal antibodies, 19-9, CSLEX-1, and DU-PAN-2 (Fig. 10). When CSLEX-1 or DU-PAN-2 was used as competitor in SPan-1 or YPan-1 double determinant radioimmunoassays at concentrations of up to 2 mg/ml protein, no specific inhibition of the binding of radiolabeled SPan-1 or YPan-1 to xenograft mucin was seen. 19-9 antibody, however, does inhibit binding by SPan-1 (Table 3).

**DISCUSSION**

The SPan-1/YPan-1 antigens from SW-1990 spent medium are high density glycoproteins which have molecular weights greater than 200,000 even after dissociating treatments such as 4 M guanidine hydrochloride and heating in the presence of SDS and \( \beta \)-mercaptoethanol. While high molecular weight carbohydrate containing substances secreted by pancreatic cancer cells may include proteoglycans in addition to mucins, the SPan-1/YPan-1 antigens are not affected by proteoglycan hydrolizing enzymes and chemicals such as chondroitinase ABC, hyaluronidase, and nitrous acid. Furthermore, the density of the SPan-1/YPan-1 antigens, 1.4–1.5 g/ml, while much higher than that of serum-type glycoproteins and of carbohydrate-free proteins, is lower than that typically found for proteoglycans (28). Most of the \(^3\H\)glucosamine labeled high

| **Table 2 Inhibition by wheat germ agglutinin** |
|-----------------|-----------------|-----------------|
| Protein (\( \mu \)g) | IgM control | YPan-1 | SPan-1 | 19-9 |
| 0.02 | 5557 ± 209 | 6252 ± 540 | 5329 ± 669 | 5579 ± 372 |
| 0.10 | 5979 ± 810 | 6576 ± 275 | 4859 ± 264 | 5646 ± 459 |
| 0.5 | 4812 ± 521 | 6088 ± 71 | 1132 ± 182 | 2115 ± 249 |
| 2.0 | 4943 ± 613 | 4852 ± 593 | 724 ± 236 | 852 ± 196 |

*Mean cpm ± SD; n = 3.
molecular weight glycoprotein in medium is immunoprecipitable with either SPan-1 or YPan-1. Moreover, the glucosamine labeled molecules are susceptible to Pronase digestion and reductive β-elimination. These results indicate that both the SPan-1 and YPan-1 antigenic determinants are carried on mucins. The antigens of B72.3 (29), 19-9 (1), and DU-PAN-2 (30) have previously been shown to be mucins by experiments similar to those presented here.

Binding of SPan-1 and YPan-1 to mucin is extremely sensitive to periodate oxidation and requires sialic acid as indicated by neuraminidase and WGA sensitivity. Denaturation of the protein by boiling or by reduction and alkylation had no effect. Pronase digestion or β-elimination resulted in YPan-1 immunoreactivity only for the xenograft antigen, probably because of the higher level of antigen in xenograft preparations. The marked reduction of the ability of isolated mucin oligosaccharides to block YPan-1 and SPan-1 immunoreactivities (Fig. 8) may be due to their having lower binding affinities for isolated oligosaccharides than for intact mucins, as has been reported (31) for other antibodies. The above results suggest that the SPan-1 and YPan-1 antibody binding sites on mucin molecules are composed mainly of carbohydrates and require sialic acid. Attempts to further characterize their epitopes with lectins were unsuccessful. This may, in part, be due to steric hindrance of sialic acids or neighboring oligosaccharides which prevent access of the lectins to their appropriate binding sites. Although SPan-1 and YPan-1 determinants are distinct, they can be found on the same mucin molecules as shown by heterologous double determinant radioimmunoassays. The two immunoreactivities could not be dissociated by WGA affinity column chromatography. The possible existence of SPan-1 or YPan-1 enriched populations of mucins is presently being investigated by means of SPan-1 and YPan-1 antibody affinity columns.

An increasing number of sialic acid requiring monoclonal antibodies have been reported to be cancer associated. These include B72.3 (32), CSELEX-1 (33), DU-PAN-2 (34), OC 125 (35), and 19-9 (36). The SPan-1 and YPan-1 antigenic determinants are distinct from those of CSELEX-1 and DU-PAN-2 since the latter antibodies do not inhibit SPan-1 or YPan-1 binding to SW-1990 xenograft mucins. SPan-1 and YPan-1 can also be distinguished from B72.3 in that SPan-1 has a 95% (10) and YPan-1 a 81% reactivity (6) with normal pancreatic tissue while B72.3 has little if any reactivity with most adult normal tissues including pancreas (32). OC 125 is distinct from SPan-1 and YPan-1 in that its antigenic determinant is heat sensitive (37). On the other hand, 19-9 does compete with SPan-1 for binding to SW-1990 mucins. Thus, the SPan-1 epitope is similar to that of 19-9. However, there is evidence that 19-9 and SPan-1 epitopes are not identical. While 19-9 does not react with colonic cancer tissues from patients with the Lewis*, Lewis’ negative phenotype, SPan-1 does (8). Experiments are in progress to isolate specific oligosaccharides from xenograft mucins which bind to YPan-1 or SPan-1 in order to obtain more details of the epitopes of these two antibodies and to determine how the antibody binding site of SPan-1 relates to that of 19-9 and of other antibodies similar to 19-9 such as C-50 (38).

Several of the mucin reactive antibodies which have a high degree of specificity for malignancy when assayed in serum show less specificity when pancreatic tissues are examined. This is the case with SPan-1 (10), 19-9 (36), and DU-PAN-2 (39). This suggests that although the epitopes themselves may not be unique to malignancy, their appearance in high levels in the bloodstream is (40). At present, little is known about the mechanisms or factors producing these high levels. It may be due to increased synthesis and/or release, or changes in the mode of release, for example, directly into the bloodstream (basolaterally rather than apically into the duct lumen), resulting from loss of polarity of cancer cells or to changes in the way such molecules are degraded. Because mucins isolated from SW-1990 carry several potentially clinically useful epitopes such as SPan-1, YPan-1, 19-9, and DU-PAN-2, the SW-1990 cell in culture and in vivo would offer an ideal model system for the study of the factors regulating the biosynthesis and release of such pancreatic cancer associated products.

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